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Abstract ☐ The preparation of 2-[*N*-(ethyl)-(*N*-β-hydroxyethyl)]aminoethyl 2,2-diphenylpropionate (1), a metabolite of aprophen [2-diethylaminoethyl 2,2-diphenylpropionate] is described. Hydrolysis of [2-(2-chloroethyl)ethylamino]ethyl acetate hydrochloride (2) in a basic solution, followed by acidic pH adjustment, gave the ethylcholineaziridinium ion (3) that upon treatment with 2,2-diphenylpropionic acid produced 1 in a 56% yield. Synthetic 1 was found to possess antimuscarinic activities, but was ~10-fold less potent than the parent compound aprophen.

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RESEARCH ARTICLES

Synthesis and Antimuscarinic Activity of 2-[N-(Ethyl)-(N-β-hydroxyethyl)]aminoethyl 2,2-Diphenylpropionate, a Metabolite of Aprophen

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Received January 28, 1992, from the *Applied Biochemistry Branch, Walter Reed Army Institute of Research, Washington, DC 20307-5100, and the §Division of Biochemistry and Biophysics, Center for Biologics Evaluation and Research, Food and Drug Administration, Bethesda, MD. Accepted for publication October 29, 1992. ‡Permanent address: Israel Institute of Biological Research, P.O. Box 19, Ness-Ziona, Israel.

Abstract □ The preparation of 2-[N-(ethyl)-(N-β-hydroxyethyl)]aminoethyl 2,2-diphenylpropionate (**1**), a metabolite of aprophen [2-diethylaminoethyl 2,2-diphenylpropionate], is described. Hydrolysis of [2-(2-chloroethyl)ethylamino]ethyl acetate hydrochloride (**2**) in a basic solution, followed by acidic pH adjustment, gave the ethylcholineaziridinium ion (**3**) that upon treatment with 2,2-diphenylpropionic acid produced **1** in a 56% yield. Synthetic **1** was found to possess antimuscarinic activities, but was ~10-fold less potent than the parent compound aprophen.

Aprophen (2-diethylaminoethyl 2,2-diphenylpropionate) is a potent spasmolytic and cholinolytic drug.[1,2] We previously demonstrated[3] that after intravenous administration of aprophen to rats, the primary metabolic product found in serum is desethylaprophen (2-ethylaminoethyl 2,2-diphenylpropionate). However, after oral intubation, the principal metabolite of aprophen in rat urine is the monohydroxy compound[4] 2-[N-(ethyl)-(N-β-hydroxyethyl)]aminoethyl 2,2-diphenylpropionate (**1**). Whereas the synthesis of aprophen is straightforward and has been described elsewhere,[5,6] the preparation of the hydroxylated metabolite of aprophen, **1**, has not been reported. Also, because a standard is required for quantification of this metabolite of aprophen, a facile two-step synthesis of **1** is reported here.

Experimental Section

Materials and Methods—^{1}H NMR spectra were recorded with a Varian XL-300FT NMR spectrometer, and all values reported in parts per million downfield from an internal standard of tetramethylsilane. Chemical ionization mass spectra, with NH_3 as the reagent gas, were recorded on a Finnigan 1015D mass spectrometer. A 100 × 8 mm i.d. Radial-Pak, type B, 10-μm silica cartridge (Waters Associates, Milford, MA) with a model ALC/GPC-204 liquid chromatograph (Waters Associates) was used for high-performance liquid chromatography (HPLC) analyses. Thin-layer chromatography (TLC) was performed on fluorescent gel polygram SiL-G/UV 254 plates. Spots were visualized by UV light and I_2 vapors. [2-(2-Chloroethyl)ethylamino]ethyl acetate hydrochloride (**2**) was obtained from Research Biochemicals Inc., Natick, MA, and was used as received.

Chemical Synthesis of 2-[N-(Ethyl)-(N-β-hydroxyethyl)]aminoethyl 2,2-Diphenylpropionate (1)—A solution of 2-[2-(chloroethyl)-ethylamino]ethyl acetate hydrochloride (2; 109 mg, 0.5 mmol) in 10 mL of distilled water was stirred for 5 min in an ice-water bath. Sodium hydroxide (0.5 N) was added to this solution until the pH reached 11.00 ± 0.05. Next, the ice-water bath was replaced with a water bath (25 °C), and additional NaOH (0.5 N) was added in a dropwise manner with stirring every 3–5 min to maintain the pH at 11.00 ± 0.05. After ~2 h, when NaOH was no longer consumed, the reaction mixture was once again cooled in an ice-water bath. HCl (0.1 N) was used to lower the pH to 7.4, and then a solution of 2,2-diphenylpropionic acid (100 mg, 0.44 mmol) in 5 mL of 10% $NaHCO_3$ was added; this was followed by the addition of 10 mL of ethyl acetate.

The resulting two-phase reaction mixture was slowly stirred at room temperature for 24 h. Then, the organic phase was separated and retained, and the aqueous phase was extracted again with ethyl acetate (3 × 10 mL). The combined organic extracts were washed with a saturated NaCl solution (10 mL), dried ($MgSO_4$), and evaporated to give 155 mg of a colorless viscous oil, which was found by HPLC analysis to be contaminated with 2,2-diphenylpropionic acid (~25%). The crude mixture was then dissolved in 2 mL of HCl (0.1 N) and extracted with ethyl acetate (10 mL). The acidic aqueous solution was neutralized with solid sodium carbonate and extracted again with ethyl acetate (3 × 10 mL), dried ($MgSO_4$), and evaporated to give 85 mg of **1** (56% yield) as a colorless viscous oil; TLC (silica, 5% MeOH:$CHCl_3$): one spot; R_f, 0.3; MS (CI/NH_3): m/z 342 (MH^+); ^{1}H NMR ($CDCl_3$): δ 0.92 (t, 3H J = 7.1 Hz), 1.93 (s, 3H), 2.48 (q, 2H, J = 7.1 Hz), 2.51 (t, 2H, J = 5.3 Hz), 2.70 (t, 2H, J = 5.6 Hz), 3.43 (t, 2H, J = 5.3 Hz), 4.21 (t, 2H, J = 5.6 Hz), 7.30 (m, 10H).

Anal.—Calcd for $C_{21}H_{27}NO_3$: C, 73.88; H, 7.95; N, 4.11. Found: C, 73.84; H, 8.01; N, 4.05.

The hydrochloride salt of **1** was prepared from etheral hydrogen chloride. Recrystallization from ethyl acetate gave needles with an mp of 131 °C.

Anal.—Calcd for $C_{21}H_{28}NO_3Cl$: C, 66.75; H, 7.41; N, 3.71. Found: C, 66.66; H, 7.43; N, 3.64.

Pharmacological Assays—To determine the antimuscarinic activity of **1**, a series of biological tests was performed. These assays assessed the ability of **1** to function as an muscarinic antagonist by (*a*) blocking the contraction of acetylcholine-stimulated guinea pig ileum, (*b*) blocking the release of α-amylase from pancreatic acinar cells stimulated by carbachol, and (*c*) by inhibiting the binding of [^{3}H]N-methylscopolamine to the muscarinic receptor of guinea pig ileum.

α-Amylase Secretion from Pancreatic Acinar Cells—Pancreatic acini were prepared from male Sprague-Dawley rats by the method by Gardner and Jensen[7] as modified by Gordon and Chiang.[8] Briefly, the cells were incubated (3×) with collagenase and then suspended in 16 mL of Dulbecco's minimal essential medium containing 0.2% albumin, 0.01% trypsin inhibitor, and 0.09% theophylline. The medium was aerated with 100% O_2 and diluted fivefold before use. Viability by trypan blue exclusion was >99%. Acinar cells were incubated with 10^{-5} M carbachol and varied concentrations of each compound to be tested in a total volume of 0.5 mL. α-Amylase secreted from the acinar cells was determined with a Pharmacia Phadebas kit (Pharmacia Diagnostics, Piscataway, NJ).

Acetylcholine-Induced Contraction of Guinea Pig Ileum—Distal ileum was obtained from male albino guinea pigs (350–500 g), and a segment ~20 mm in length was suspended in a 10-mL organ bath in oxygenated Krebs-Ringer solution[9] and maintained at 37 °C. Isometric contractions were recorded with a free-displacement transducer (Harvard Apparatus, Natick, MA) set at 1 g of tension. After an equilibrium period of 45 min, acetylcholine (ACh) was added to the bath, allowed to react for 1 min, and then washed out. The tissue was allowed 5 min to recover

Table I—Antimuscarinic Activities of 1 and Related Compounds

Compound	Ileum Contraction		Inhibition of α-Amylase Release	[^{3}H]NMS Binding
	pA_2	K_B, M	K_i, M	K_i, M
1	7.7 ± 0.1	2.2×10^{-8}	$2.3 \pm 1.0 \times 10^{-8}$	$4.5 \pm 0.5 \times 10^{-7}$
Aprophen	8.5 ± 0.1	3.1×10^{-9}	$1.7 \pm 0.7 \times 10^{-9}$	$3.8 \pm 0.8 \times 10^{-8}$
Atropine	8.7 ± 0.1	2.0 ± 10^{-9}	$1.6 \pm 1.1 \times 10^{-9}$	$5.0 \pm 0.8 \times 10^{-9}$
Desethylaprophen	6.7 ± 0.1	2.2×10^{-7}	$7.8 \pm 2.7 \times 10^{-8}$	$6.3 \pm 1.8 \times 10^{-8}$

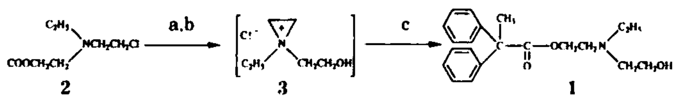
affinity constant (pA_2) values [(pA_2) = $-\log(K_B)$], measuring the affinity of an antagonist for muscarinic receptors, were calculated with a computer program for the Schild plot.[10]

Binding Assays—Ileum was minced, mixed with an equal volume (w/v) of 37.5 mM Tris-HCl buffer (12.5 mM $MgCl_2$, pH 7.4), and homogenized at 4 °C. The homogenate was centrifuged at 10 000 × *g* for 20 min at 4 °C, and the resulting pellet was resuspended in the same volume of buffer and centrifuged again. The pellet was resuspended in buffer and frozen in aliquots at −30 °C. [^{3}H]*N*-Methylscopolamine ([^{3}H]NMS) binding to homogenized guinea pig ileum was carried out in 96-well tissue culture plates at 25 °C in a final volume of 0.25 mL. The concentration of [^{3}H]NMS was ~2 nM, and nonspecific binding was determined in the presence of 10 μM atropine.[11] To assess binding, the homogenized ileum plus or minus test compound was added to 96-well plates (Costar, Cambridge, MA), and the reaction was initiated by the addition of [^{3}H]NMS. The plates were then placed on a rocking platform for 1 h at 25 °C. The reaction was terminated by collecting the protein on glass fiber filters and rapidly washing with ice-cold phosphate buffer (50 mM potassium phosphate, pH 7.3) with an automated cell harvester (Cambridge Technology, Cambridge, MA). The dried glass fiber filter disks were placed into scintillation vials, and 8 mL of Ready Value (Beckman Instruments, Fullerton, CA) was added prior to scintillation counting.

Results and Discussion

We report for the first time the synthesis of **1**. Treatment of **2** with aqueous NaOH, followed by addition of HCl (pH 7.4), generated the ethylcholineaziridinium ion[6] (**3**). Then, **3** was reacted with the sodium salt of 2,2-diphenylpropionic acid to produce the final product **1** (Scheme I). The structure of **1** was confirmed by spectroscopic and elemental analyses. TLC and normal-phase HPLC[4] were used to verify **1** as a metabolite of aprophen.

We showed previously that [^{14}C]aprophen is rapidly cleared from the blood following intravenous injection into rats with a half-life of 4 min.[12] At the present time, it is unknown whether metabolites of aprophen contribute significantly to the pharmacological profile of aprophen. However, one metabolite, desethylaprophen, has antimuscarinic activities[12] (Table I). In this paper, we report that another metabolite of aprophen, **1**, also has antimuscarinic activity. The antimuscarinic activity of **1** was less than that of its parent compound aprophen, the aprophen metabolite desethylaprophen,[3] or atropine (Table I). The affinity constant (pA_2) for **1**, obtained by Schild plot analysis for the guinea pig ileum, was 7.7 (K_B of 2.2×10^{-8} M). The slope of the Schild plot was close to 1, suggesting competitive inhibition. In comparison, aprophen has been shown to have a pA_2 of 8.5 (K_B of 3.1×10^{-9} M), and is thus ~10-fold more potent than the metabolite.[13] Compound **1** blocked the carbachol-stimulated release of α-amylase from rat pancreatic acini, with an inhibition constant (K_i) of 2.3×10^{-8} M, which is ~10-fold less than the K_i obtained for aprophen (K_i for aprophen is 1.7×10^{-9} M). Compound **1** inhibited the binding of [^{3}H]NMS to the ileum homogenates, with a K_i of 4.5×10^{-7} M, which is also ~10-fold less than that for aprophen. These in vitro results indicate that the antimuscarinic properties of **1** were attenuated ~10-fold compared with those of aprophen.



2 3 1

Scheme 1—Synthetic pathway for 2-[*N*-(ethyl)-(*N*-β-hydroxyethyl)]aminoethyl 2,2-diphenylpropionate (**1**). Key: (**a**) NaOH (0.5 N), pH 11.0, 2 h; (**b**) HCl (0.1 N), pH 7.4; (**c**) $Ph_2C(CH_3)COO^-/NaHCO_3/H_2O$.

In general, there was good agreement among the trends of potency observed in the three assays of antimuscarinic activity. Atropine and aprophen were the most potent compounds, whereas the metabolites desethylaprophen and **1** were the least potent. Atropine showed no subtype specificity between the muscarinic receptors in the three assays because the K_i values were similar. There was only an ~10-fold difference between K_i values for **1** among the assays. Thus, **1** is most likely not a subtype-selective compound,[8] and neither are aprophen or desethylaprophen. However, β-hydroxylation of one of the ethylamino groups of aprophen, yielding **1**, may have a pronounced effect on the basicity of the nitrogen, making protonation of this nitrogen less likely at physiological pH. This could increase the partition coefficient of **1** into a lipophilic milieu, and hence render it more likely to cross the blood–brain barrier. Thus, **1** might be a more effective antidote to organophosphate poisoning than aprophen in vivo.

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